Förster resonance energy transfer (FRET) is a mechanism where energy is transferred from an excited donor fluorophore to adjacent chromophores via non-radiative dipole-dipole interactions. FRET theory primarily considers the interactions of a single donor-acceptor pair. Unfortunately, it is rarely known if only a single acceptor is present in a molecular complex. Thus, the use of FRET as a tool for measuring protein-protein interactions inside living cells requires an understanding of how FRET changes with multiple acceptors. When multiple FRET acceptors are present it is assumed that a quantum of energy is either released from the donor, or transferred to only one of the acceptors present. The rate of energy transfer between the donor and each specific acceptor (k_{DA}) can be measured in the absence of other acceptors, and these individual transfer rates can be used to predict the ensemble FRET efficiency. The generality of this approach was tested by measuring the ensemble FRET efficiency in two constructs, each containing a single fluorescent-protein donor (Cerulean) and either two or three acceptors (Venus). FRET transfer rates between individual donor-acceptor pairs were measured by systematically introducing point mutations to eliminate the chromophores of the other acceptors. We find that the amount of FRET with multiple acceptors is significantly greater than predicted by the sum of the individual transfer rates. We conclude that either an additional energy transfer pathway exists when multiple acceptors are present, or that a theoretical assumption that the prediction calculation is based on is incorrect. These possibilities will be discussed.

3026-Pos

Fluorescence Fluctuation Spectroscopy in the Presence of Hydrodynamic Flow to Determine Protein Stoichiometry at Ultra Low Concentrations

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Fluorescence fluctuation spectroscopy (FFS) provides information about transport parameters, concentration, and interactions of fluorescently labeled molecules. One important FFS parameter is brightness which provides information about the stoichiometry of protein complexes. Here we consider the application of FFS to large protein complexes, such as viruses. FFS measurements of such systems typically require very long data acquisition times due to the low concentration and slow diffusion of the large particles. In order to overcome this drawback we apply hydrodynamic flow, which results in an increased flux of particles passing through the optical observation volume. This technique significantly reduces the data acquisition time of brightness experiments, while extending brightness analysis to femtomolar concentrations. The technique was developed using a test system of fluorescently labeled microspheres flowing through microfluidic channels to investigate the effect flow speed, particle size and brightness on FFS parameters. Finally this technique was extended to determine the copy number of fluorescently labeled Gag protein in viral-like particles present in cell medium. This work is supported by NIH grant R01GM064589.

3027-Pos

Characterization of Conjugated Protein by Molecular Brightness and Mass Spectrometry

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We have previously demonstrated characterization of antigen-antibody interactions with brightness analysis in fluctuation spectroscopy. In these experiments, the antigen studied was labeled with only a single fluorophore. In practice, it may not be straightforward to produce antigen with only a single fluorophore and random conjugation with a dye becomes the simplest approach. To use conjugated protein for single molecule studies, the distribution of conjugate must be understood. Here we introduce a method to evaluate conjugation of proteins using mass spectrometry. We define a mass spread function which describes the distribution of conjugate on a given protein. We show that convolution of this mass spread function with a protein's measured mass spectrum predicts the mass spectrum of conjugated protein. Application is shown using a highly glycosylated antibody with a low amount of incorporated conjugate. For measurement in solution, we use time integrated fluorescence cumulant analysis to characterize conjugated protein in terms of the molecular brightness. We then use brightness analysis to measure concentrations of free and bound conjugated protein in the presence of antibody.

3028-Pos

Combined Optical Tweezers and Fluorescence Microscopy for Single Molecule Experiments

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Optical traps (OT) and single molecule fluorescence (SMF) find use in single molecule biology [1,2]. OT allows manipulation and force spectroscopy of biological macromolecules. Unfortunately both the conformational state of the molecule and force localization on the molecular complex are unresolved in space. SMF characterizes the position of the fluorophore label, yielding the change in shape and conformational state of the labeled molecule. Correlating the force response and label tracking data, the relationship between changes in conformational state and related force are resolved [3]. A combined OT and SMF instrument (OT-SF) allow studying the operational principle of biological molecular motors essential to life.

We implemented SMF imaging into an existing optical tweezer instrument [4]. A diode pumped solid state laser excites fluorescence (Coherent Sapphire 50 mW, 488 nm). Video capture is realized with an intensified CCD camera (Qimaging QICAM Fast). A fluorescence emission filter (Chroma HQ535/30m) maximizes the SNR of fluorescence detection, by maximizing the optical density at the wavelengths corresponding to the trapping and detection lasers. A microscope TIRF objective (Nikon CFI Plan Apo 100X TIRF) facilitates localized excitation in the sample chamber, which reduces the background signal.

Proof-of-principle concurrent SMF imaging and OT micromanipulation of SYBR Gold stained DNA constructs is presented. The proof is presented in the form of a nanoscale video with fluorophore position and forces displayed in real-time. The DNA is bound to optically trapped dielectric beads in a dumbbell configuration.

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3029-Pos

Hyperspectral Line Scanning Microscopy for High-Speed Multicolor Quantum Dot Tracking

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One of the fundamental goals in observing protein-protein interactions on the cell membrane is in achieving nanometer scale spatial resolution along with temporal resolution sufficient to study live cell behavior. Traditional fluorescence microscopy methods have been unsuccessful in studying these interactions due to the diffraction limit with visible light. Single particle tracking techniques using quantum dots have provided single particle localizations to well below the diffraction limit, however, clustering of multiple particles limits the unique identification and thus tracking of individual particles throughout the (possibly dynamic) clustering process. This problem can be solved by tracking multiple quantum dot colors using a high-speed hyperspectral microscope which provides the necessary spatial, spectral, and temporal performance.

We describe a line scanning hyperspectral microscope that uses a prism spectrometer and a fast EMCCD camera to achieve 30 frames per second with 128 spectral channels. We present the optical setup, instrument control and display software and preliminary studies of multi-color quantum dot single particle tracking.

3030-Pos

A Time Resolved Fluorescence Spectrometer with Sub-Millisecond Data Acquisition Time

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We have developed a high-throughput time-resolved fluorescence spectrometer capable of recording a high-resolution time-domain (**sub-nanosecond**) fluorescence decay, with high S/N every 100 μs. Coupled with a conventional stopped-flow rapid mixer, this technology has allowed us to measure changes in time-resolved fluorescence decays occurring during the course of a millisecond-resolved biochemical transient experiment. Most instruments used in fluorescence-based kinetic studies are limited to detecting a single fluorescence intensity signal on the millisecond time scale. While this type of fluorescence intensity-based measurement is informative, it provides scant information compared to a full sub-nanosecond resolved fluorescence decay-which is exquisitely sensitive to the structure, dynamics, and interactions